Synthesis and Germicidal Activity of Halogenated Salicylanilides and Related Compounds

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Synthesis and germicidal activity in the presence of detergents are reported for halogenated derivatives of the following classes of compounds: salicylanilides, homologs of salicylanilide, salicylanilides with substituents other than halogen or alkyl groups, other amides of salicylic acid, anilides of other acids, and Schiff's bases of salicylaldehyde. The anilides were prepared by first halogenating the acid or aniline in the desired manner and then reacting these products to form the amide linkage. In some cases, when the position of the incoming halogen would be unambiguous, the anilides were further halogenated to form new products. Bactericidal effectiveness and skin substantivity of the products were evaluated using filter paper and skin disk substantivity tests in the presence of soap or detergent. High activity in soap was shown by salicylanilides halogenated in these positions: 4',5; 2',4',5; 3,4',5; 3',4',5; 4,4',5; 2',3,4',5; 3,3',4',5; 2',3,4',5,5'. Certain homologs and nitro-derivatives also possessed high activity.

THE GERMICIDAL properties of the halogenated salicylanilides have received considerable attention (1–19). Few details are available, however, on the activity of these materials in the presence of soap and no data have been published on their substantivity to skin. Ninety-two halogenated salicylanilides and related compounds have now been synthesized and tested for germicidal activity and skin substantivity in the presence of soap or detergent. The details of synthesis and the results of germicidal evaluation in the filter paper disk and skin disk substantivity tests are presented here.

EXPERIMENTAL

The compounds prepared are listed in Table I. The general preparative procedures are illustrated with specific examples. Analytical data and literature references for individual compounds are shown in Table I.

1a. **3,4',5-Tribromosalicylanilide.**—A slurry of 23.7 Gm. of 3,5-dibromosalicylic acid and 13.8 Gm. of 4-bromoaniline in 200 ml. of chlorobenzene was refluxed with 5.4 Gm. of phosphorus trichloride and 0.5 Gm. of aluminum chloride for four hours and then filtered hot. The filtrate deposited 28 Gm. (78%) of 3,4',5-tribromosalicylanilide, melting at 222–226°.

1b. **2',3,4',5-Tetrabromosalicylanilide.**—A slurry of 10 Gm. of dibromosalicylic acid and 9 Gm. of 2,4-dibromoaniline in 200 ml. of chlorobenzene was refluxed with 1.5 ml. of phosphorus trichloride for three hours and then decanted hot. The cold solution deposited 12 Gm. (68%) of 2',3,4',5-tetrabromosalicylanilide, melting at 203–208°.

2. **5-Bromo-4'-chlorosalicylanilide.**—A slurry of 6 Gm. of 4'-chlorosalicylanilide (melting at 164–168°; Cl found, 13.93%) in 60 ml. of acetic acid was

treated with 4 Gm. of bromine and then heated until fumes of hydrogen bromide appeared. The next day 7 Gm. (86%) of 5-bromo-4'-chlorosalicylanilide, melting at 238–243°, was collected.

3. **2-Acetoxy-4',5-dibromobenzanilide.**—A solution of 18 Gm. of 4',5-dibromosalicylanilide was prepared in 0.5 L. of water containing 6 Gm. of sodium hydroxide. Some suspended particles were filtered off and the solution treated with 6 Gm. of acetic anhydride. The solid was filtered and washed thoroughly with chloroform. The chloroform was separated, concentrated, and cooled, whereupon 5 Gm. of 2-acetoxy-4',5-dibromobenzanilide, melting at 155–164°, was collected.

4. **4-Bromo-N(2-hydroxybenzylidene) Aniline.**—Ethanol solutions of 6 Gm. of salicylaldehyde, and 8.6 Gm. of 4-bromoaniline were prepared separately and mixed. The Schiff's base precipitated and was filtered. Recrystallization from alcohol gave 10 Gm. (72%) of yellow platelets melting at 110–112°.

5. **3,4',5-Tribromo-2-aminobenzanilide.**—A solution of 2.65 Gm. of 2-aminobenzanilide in 100 ml. of acetic acid and 3.8 Gm. of triethylamine was treated with 6 Gm. of bromine. The next day the product was collected. After two recrystallizations from alcohol it weighed 1 Gm. (18%) and melted at 215–218°.

DISCUSSION

When salicylanilide or a pure substituted salicylanilide is reacted with bromine the compounds which result will depend on the relative reactivities of the unsubstituted sites in the salicylanilide molecule. If the reaction involves only one mole of bromine and if there is one site in the molecule much more reactive than any other, a relatively pure compound can be obtained.

A number of compounds listed in Table I were prepared by bromination of halogenated salicylanilides (see Experimental, method 2). Sixteen compounds synthesized in this manner are listed in Table II. The purity of these materials was estimated from the sharpness of the melting points (M. P.) and from the elemental analyses (Anal.); their identities were established by a comparison of their melting points and infrared spectra (IR) with those of known com-

TABLE I.—SYNTHETIC MATERIALS RELATED TO SALICYLANILIDE

Substituents and Positions	Method of Prepn. and Purifn.[a,b]	Melting Point, °C. Found	Literature	Analysis Elem.	Calcd.	Found	Lit. Refs.
Halogenated Salicylanilides[c]							
4'-Br	1a Pur Recr. P	173–175	...	Br	27.35	26.95	(5)
5-Br	1b Recr. A	225–227	222 (21)	Br	27.35	26.92	(5, 21)
2',5-Br$_2$	1b Recr. P	189–191	...	Br	43.08	43.04	...
3,4'-Br$_2$	1b Recr. TA	214–216	...	Br	43.08	42.86[d]	...
3,5-Br$_2$	1a Recr. T	138–140	139–140 (22)	Br	43.08	42.98	(5, 22)
3',5-Br$_2$	1a Recr. P	222–225	...	Br	43.08	42.76	...
4',5-Br$_2$	1b Recr. APP	240–241	233–234 (8)	Br	43.08	43.25	(5–8, 13)
2',3,5-Br$_3$	1b	178–179	...	Br	53.28	53.16	
2',4',5-Br$_3$	1a Recr. PAP	233–236	...	Br	53.28	52.95[d]	(13)
3,3',5-Br$_3$	1b Recr. P	155–158	...	Br	53.28	53.38	...
3,4',5-Br$_3$	1b Recr. PAP	225–227	...	Br	53.28	52.91	...
3',4',5-Br$_3$	1b Recr. A	252–254	...	Br	53.28	52.82	...
4,4',5-Br$_3$	1b Recr. AP	258–260	...	Br	53.28	52.89	...
2',3,4',5-Br$_4$	1b Recr. PP	206–208	...	Br	60.44	60.46	...
3,3',4',5-Br$_4$	2 Recr. P	192–194	...	Br	60.44	59.52	...
2',3,4',5,5'-Br$_5$	1b Recr. A	214–216	...	Br	65.74	65.28	...
2'-Cl	1b Recr. PA	167–168	168–169 (23)	Cl	14.32	14.15	(23)
4'-Cl	1a Recr. P	166–168	167–168 (8)	Cl	14.32	13.93	(5, 8, 23–25)
5-Cl	1a Recr. AP	209–211	203–204 (27) 207 (28)	Cl	14.32	14.41	(5, 15, 26–28)
2',5-Cl$_2$	1b Recr. P	230–232	228 (29)	Cl	25.13	24.58	(5, 28, 29)
3',4'-Cl$_2$	1b	213–216	...	Cl	25.13	25.35[d]	(9)
3,5-Cl$_2$	1a[e] Recr. P	133–135	134–135 (30) 134–135 (22)	Cl	24.13	24.50	(5, 19, 22, 30)
4',5-Cl$_2$	1b Recr. A	231–232	215–216	Cl	25.13	24.58	(13, 15, 27)
2',4',5-Cl$_3$	1b	216–219	218–219 (11)	Cl	33.60	33.07	(2, 3, 5, 11, 13, 15)
3,3',5-Cl$_3$	1b Recr. PA[e]P	135–150	...	Cl	33.60	33.11	...
3,4',5-Cl$_3$	1b Recr. P	190–192	188–189	Cl	33.60	33.00	(5, 19, 30)
3',4',5-Cl$_3$[f]	1a Recr. PA	246–248	246–248	..	...	...	(1–3)
2',3,4',5-Cl$_4$	1b Recr. P	180–181	174–175 (27)	Cl	40.40	40.00	(5, 27)
3,3',4',5-Cl$_4$	1b Recr. B	149–151	162 (11)	Cl	40.40	40.22	(11, 17)
2'-Br-5-Cl	1b Recr. P	179–182	178 (8)	[g]	1.014	1.007	(5, 8)
4'-Br-5-Cl	1a Recr. PPA	224–226.5	222 (8)	[g]	1.014	0.995	(5–8, 13)
5-Br-2'-Cl	2 Recr. PA	202–203	...	[g]	1.014	1.008	(5)
5-Br-4'-Cl	2 Recr. P	238–243	241–244 (8)	[g]	1.014	1.005	(5–8)
2',4'-Br$_2$-5-Cl	1b Recr. PA	225–226	...	[g]	1.280	1.251	(5, 13)
3,4'-Br$_2$-5-Cl	1a Recr. A	210–214	...	[g]	1.280	1.272	...
3,5-Br$_2$-2'-Cl	1b Recr. P	167–169	...	[g]	1.280	1.242[d]	(31)
3,5-Br$_2$-3'-Cl	1a Recr. AA	160–162	...	[g]	1.280	1.250	(31)
3,5-Br$_2$-4'-Cl	1a Recr. A	199–203	200 (8)	[g]	1.280	1.274	(6–8)
4',5-Br$_2$-2'-Cl	1b Recr. P	236–241	...	[g]	1.280	1.284	...
4',5-Br$_2$-3'-Cl	1a Recr. P	220–226	...	[g]	1.280	1.276	...
3-Br-4',5-Cl$_2$	1b Recr. P	187–190	(8)	[g]	1.314	1.301	(8)
4'-Br-3,5-Cl$_2$	1a Recr. P	209–212	...	[g]	1.314	1.288	...
5-Br-3,4'-Cl$_2$	1b Recr. A	195–200	...	[g]	1.314	1.312	...
5-Br-3',4'-Cl$_2$	1b Recr. P	235–238	...	[g]	1.314	1.314	...
3,4',5-Br$_3$-3'-Cl	2 Recr. A	178–181	...	[g]	1.459	1.449	...
3,5-Br$_2$-2',4'-Cl$_2$	1b Recr. P	179–193[h]	178–179 (11)	[g]	1.505	1.505	(11, 17)
3,5-Br$_2$-2',5'-Cl$_2$	1a Recr. P	156–159	...	[g]	1.505	1.475	...
3,5-Br$_2$-3',4'-Cl$_2$	1b Recr. A	171–172.5	176–178 (11)	[g]	1.505	1.481	(11, 17)
4'-Br-3,3',5-Cl$_3$	2[i] Recr. P	172–175	...	[g]	1.562	1.544	...
3,4',5-Br$_3$-2',5'-Cl$_2$	1b Recr. A	187–189	...	[g]	1.638	1.622	...
3,5-Br$_2$-4'-F	1b[j] Recr. AA	183–193	...	Br	41.08	40.59	...
Halogenated Sulfur Derivatives of Salicylanilide							
3,5-Br$_2$-2'-SO$_2$NH$_2$	1b Recr. A	228–231	...	Br	35.50	34.93	...
3,4',5-Br$_3$-2'-SO$_2$NH$_2$	1b Recr. PP	249–251	...	Br	45.32	44.15	...
3,5-Br$_2$-4'-SO$_2$NH$_2$	1b	263 decompn.	...	Br	35.50	34.95	...
3,5-Br$_2$-2'-SH[m]	1b Recr. P	171–179	...	Br	39.75	42.05	...
Halogenated Homologs of Salicylanilide							
3,4',5-Br$_3$-N-CH$_3$	2 Recr. P	178–180	...	Br	51.67	51.22	...
4' Br-3-CH$_3$	1b Recr. P	150–154	...	Br	26.10	26.09	...
3',5-Br$_2$-3-CH$_3$	1b Recr. PP	136–147	...	Br	41.51	41.64	...
4',5-Br$_2$-3-CH$_3$	2 Recr. P	158–159	162–163 (34)	Br	41.51	41.41	(34)
3',4',5-Br$_3$-3-CH$_3$	2[k] Recr. PP	211–213	...	Br	51.67	50.72	...
5-Br-2',4'-Cl$_2$-3-CH$_3$	1b	157–158	...	[g]	1.265	1.261	...
5-Br-2',5'-Cl$_2$-3-CH$_3$	1b	169–173	...	[g]	1.265	1.255	...
3,5-Br$_2$-3'-CH$_3$	1b Recr. P	158–164	...	Br	41.51	41.78	...
3,4',5-Br$_3$-3'-CH$_3$	2	165–169	...	Br	51.67	51.56	...
3,5-Br$_2$-4'-CH$_3$	1b	161–162 173–175	...	Br	41.51	41.73	...

TABLE I. (*continued*)

Substituents and Positions	Method of Prepn. and Purifn.[a,b]	Melting Point, °C. Found	Literature	Analysis Elem.	Calcd.	Found	Lit. Refs.
5-Br-3'-Cl-4'-CH₃	1b Recr. PA	236–238	...	g	0.972	0.969	...
3,5-Br₂-3'-Cl-4'-CH₃	1b	185–188	...	g	1.237	1.233	...
5-Br-3,4'-(CH₃)₂	2	153–163	...	Br	24.96	25.84	...
Other Halogenated Salicylanilides							
5,5'-Cl₂-2'-OCH₃	1a Recr. PA	223–224	...	Cl	22.72	22.21	...
4'-Br-4-NHCOCH₃	1b Recr. P	258–265	...	Br	22.89	20.76	...
5-Br-3',4'-Cl₂-3-C₆H₅	1b Recr. F	100–106	...	g	1.085	0.986	...
4',5-Br₂-O-Acetyl	3	155–164	...	Br	38.69	38.41	...
3',4'-Cl₂-3-NO₂	1b Recr. P	173.8–174.2	...	Cl	21.68	21.16	...
3',4'-Cl₂-5-NO₂	1b Recr. P	280–282 decompn.	...	Cl	21.68	20.95	...
5-Br-3',4'-Cl₂-3-NO₂	1b Recr. P	192.5–194	...	g	1.169	1.083	...
4-Hydroxybenzanilides							
4'-Br	1a Recr. PCP	203–213	...	Br	26.09	24.65	...
3,4'-Br₂	2 Recr. P	203–211	...	Br	43.08	40.98	...
3,5-Br₂	1b Recr. P	202–218	...	Br	43.08	43.46	...
3,4',5-Br₃	1a Recr. P	214–218	...	Br	53.28	53.01	...
Resorcylanilides							
4'-Br	1a Recr. PCC	183–186	...	Br	25.93	24.78	...
4',5-Br₂	2 Recr. D	223–225 decompn.	...	Br	41.29	41.09	...
3,4',5-Br₃	2 Recr. P	196–206	...	Br	51.45	50.95	...
N-Benzylsalicylamides							
3,5-Br₂-4'-Cl	1b Recr. C	139–142	...	g	1.237	1.224	...
3,5-Br₂-3',4'-Cl₂	1b Recr. C	157–160	...	g	1.459	1.443	...

Schiff's Bases

Amine	Aldehyde	Method	MP Found	Literature	Elem.	Calcd.	Found	Lit. Refs.
4-Aminosalicylic acid	5-Bromosalicylaldehyde	4	193–195	194 (35)	Br	23.77	24.64	(35)
4-Bromoaniline	Salicylaldehyde	4 Recr. B	110–112	...	Br	28.94	29.26	...
4-Bromoaniline	5-Bromosalicylaldehyde	4 Recr. A	173–176	...	Br	45.02	45.44	...
2-Hydroxyaniline	5-Bromosalicylaldehyde	4	186–189	...	Br	27.26	27.11	...

Other Compounds

Substituents and Positions	Method	MP Found	Literature	Elem.	Calcd.	Found	Lit. Refs.
2-Amino-3,4',5-tribromobenzanilide	5	215–218	...	Br	53.40	52.85	...
4,4'-Dibromo-3-hydroxy-2-naphthanilide	1b Recr. C	230–237 decompn.	234 (32)	Br	37.95	36.69	(32)
3,5-Dibromosalicylamide	2 Recr. B	182.5–184	182–183 (33)	Br	46.87[l]	46.62	(33)
4'-Bromo-2-mercaptobenzanilide[n]	1b[m]	238–241	...	Br	26.01	24.91	...
N,N'-Hexamethylene bis (3,5-dibromosalicylanilide)	1b Recr. P	204–224	...	Br	47.56	47.50	...

[a] Methods of preparation are numbered as in the specific examples given in the text. [b] Methods of purification include: evaporation of solvent from a solution of the product in a beaker followed by mechanical removal of the top, colored layer from the walls of the beaker (designated "Pur"), and recrystallization (Recr.) from the following solvents: P, chlorobenzene; A, acetone; B, ethanol; C, acetic acid; D, acetone and chloroform; E, ethyl acetate; F, benzene and petroleum ether; T. toluene. [c] Halogenated salicylanilides are listed in the following order: (a) bromine derivatives, from mono- to penta-brominated; (b) chlorine derivatives, from mono- to tetra-chlorinated; (c) bromochloro derivatives from di- to penta-halogenated, within each group compounds with larger number of bromine substituents are given first; (d) a bromofluorosalicyl-anilide. [d] Microanalyses performed by Schwarzkopf Microanalytical Laboratory, all other analyses were performed by Messrs. W. H. Adam, L. F. Pierro, C. N. Buffa, D. P. Prescott, R. L. Eichenlaub, and D. J. Berry at Lever Brothers Research Center. [e] Second crop, after preliminary filtration of small initial precipitate. [f] Infrared spectrum identical to commercial 3',4',5-trichlorosalicylanilide manufactured by Geigy Chemical Co. [g] Grams of silver halide precipitate per gram of sample. [h] Appeared to be dimorphic. [i] In the presence of pyridine. [j] A 25-ml. quantity of chlorobenzene used per Gm. of solid. Crude product obtained by evaporating solvent. [k] In the presence of iodine. [l] One molecule of ethanol of crystallization. [m] In anisole. [n] As the disulfide.

pounds. In some cases when the site available for bromination seemed unambiguous from previous experience, elemental analysis alone served as a basis for identification of the brominated product.

BIOLOGICAL ACTIVITY

Data from the laboratories of Geigy Chemical Co. (1–4), Knoll, A. G. (5–8), Ciba (9–10), BASF (11), Monsanto Chemical Co. (12–14), Wallace and Tiernan (15), and Ben Venue Laboratories (16), as well as several university laboratories (17–19), have illustrated the germicidal activity of a large number of halogenated salicylanilides. This work may be summarized briefly as follows: (a) Good activity (bacteriostasis of *S. aureus* 209 at 2 parts of germicide in 1,000,000) could be achieved with substitution in just one ring of salicylanilide. Maximum activity, however, was always associated with substi-

TABLE II.—BROMINATION OF SALICYLANILIDES

Substituents in Starting Material	Moles, Br_2	Solvent	Added Substituents	Method of Identification
None	1	HOAc	5-Br	IR,[a] M.P.
None	2	HOAc	5-Br 4',5-Br_2[b] 3,4',5-Br_2	IR
2'-Cl	1	HOAc	5-Br	Anal.
2'-Cl	2	HOAc	3,5-Br_2	IR, M.P.
4'-Cl	1	HOAc	5-Br	M.P.
2',5'-Cl_2	3[c]	HOAc	3,5-Br_2	IR
3',5-Br_2	1	HOAc/I_2	3-Br 50% 4'-Br 50%	M.P. M.P.
2'-Br-5-Cl	1	HOAc	Mixtures	M.P.
2',4',5-Br_3	1	aq. HOAc	3-Br	IR
2',3,5-Br_3	1	aq. HOAc	4'-Br	IR
3,3',5-Br_3	1	HOAc/I_2 long heating	4'-Br	Anal.
3,5-Br_2-3'-Cl	1	aq. HOAc	4'-Br	Anal.
3,5-Br_2-2',5'-Cl_2	1	aq. HOAc	None	IR
3,5-Br_2-2'-SO_2NH_2	1	aq. HOAc	None	Anal.
3-Me-4'-Br	1	HOAc	5-Br	Anal.
3-Me-3',5-Br_2	1	HOAc/I_2	4'-Br	Anal.
3'-Me-3,5-Br_2	1	HOAc	4'-Br	Anal.
3,4'-Me_2	1	HOAc	5-Br	Anal.

[a] We are indebted to Dr. S. Goldwasser and Mr. A. Rapisarda of this laboratory for interpretation of infrared spectrograms.
[b] Major product.   [c] Only two moles reacted.

tution in each ring. Dilution values of 1 p.p.m. and better were frequently attained. (*b*) Substitution in the electron-rich positions (*ortho* or *para* to the phenol and amide groups) yielded active compounds as did vicinal substitution (simultaneous *meta* and *para* substitution in either ring). (*c*) Great and apparently unpredictable changes in activity resulted from changing a bromine atom to a chlorine atom in a given structure.

In the present work the biological activity of the test compounds was determined in the filter paper disk (FPD) and skin disk substantivity (SDS) tests previously described (20). Ratings given in these tests have the following significance:

Filter Paper Disk Test.— + +, zone greater than 1 mm.; +, 1 mm. zone or less; ±, growth inhibition under disk; 0, no activity.

SDS Test.—Rating of 4, sharply outlined clear area free of growth (excellent activity); rating of 3, clear area of no growth with hazy periphery (good activity); rating of 2, area showing partial growth (fair activity); rating of 1, area showing growth almost equal to surrounding agar (slight activity); rating of 0, area showing growth equal to surrounding agar (no activity).

In the SDS test, six disks are judged and the results averaged numerically to two significant figures which are further rounded off as follows:

SDS TEST SCORES

Average of Six Observations	Score Tabulated
3.6–3.8	4–
3.2–3.5	3+
2.9–3.1	3

Both tests are carried out in the presence of soap or detergent. The SDS test scores depend not only on germicidal activity but also, as the name implies, on the substantivity of the test compound to skin. They might therefore be expected to differ from results previously published in the literature. Nonetheless, the three generalizations listed at the beginning of this section were, in the main, supported by

the present results. Thus, substitution in both salicylanilide rings was necessary (but not sufficient) for a score of 3 or better in the SDS test in the presence of soap. The twelve compounds which scored the maximum of 4 were characterized by *ortho* and *para* and/or vicinal substitution. Three of the twelve had only *ortho* and *para* substitution; three possessed vicinal substitution in one ring and were mono-substituted in the other; and six compounds carried *ortho* and *para* substituents in one ring and vicinal substituents in the other.

Interchange of bromine and chlorine had some effect on SDS test scores but the effect is less dramatic than occasionally found in simple dilution tests. Thus, 2',4',5-trichlorosalicylanilide scores 2+ and a bromine analog, 5-chloro-2',4'-dibromosalicylanilide, attains a score of 4. In the usual dilution tests these two compounds have been reported to differ by a factor of 500 in threshold value (5). On the other hand, three out of four 4',5-dihalosalicylanilides gave identical SDS test scores of 3+; the fourth, 5-bromo-4'-chlorosalicylanilide, scored 3–. Surprisingly, this last compound was the most active one in the dilution test (5). For the eight 3,4',5-trihalosalicylanilides the scores ranged from 2+ to 4, with six falling between 3 and 4–.

Of the ninety-two test compounds, SDS test scores of 3 or better were recorded for 34 compounds in the presence of soap, for 19 compounds in the presence of an alkaline soap/detergent bar, and for six compounds in the presence of a neutral detergent bar. While fewer compounds were tested in the presence of the latter two bar stocks, it is safe to conclude that high germicidal activity in the presence of detergents is considerably rarer than high activity in the presence of soap.

In general, high activity at 0.5% was associated with structures halogenated in the following positions: 4',5; 2',4',5; 3,4',5; 3',4',5; 4,4',5; 2',3,4',5; 3,3',4',5; 2',3,4',5,5'. Substitution by groups other than halogen had a generally depressing effect upon germicidal activity.

TABLE III.—ACTIVITY OF BACTERICIDES AT 0.5% IN SOAP OR NONSOAP DETERGENTS AGAINST *S. aureus*

Substituent and Position	FPD Test[a] in Presence of Bar Stock Type A[b]	SDS Test[a] in Presence of Bar Stock		
		Type A[b]	Type B[c]	Type C[d]
Halogenated Salicylanilides				
4'-Br	−−	0+	. . .	. . .
5-Br	−−	0+	. . .	. . .
2',5-Br₂	. . .	1	. . .	. . .
3,4'-Br₂	0	1+	. . .	. . .
3,5-Br₂	±	0+	1	. . .
3',5-Br₂	++	3−	1	. . .
4',5-Br₂	++	3+	2	1
2',3,5-Br₃	++	1+	. . .	<1
2',4',5-Br₃	++	3+	2	. . .
3,3',5-Br₃	++	2+	2	1
3,4',5-Br₃	++	3+	3+	1+
3',4',5-Br₃	++	4	3	. . .
4,4',5-Br₃	++	4	3+	1
2',3,4',5-Br₄	++	3+	3+	1+
3,3',4',5-Br₄	++	4	3+	3
2',3,4',5,5'-Br₅	++	4	. . .	2
4'-Cl	−−	0+	. . .	. . .
5-Cl	+	1+	. . .	. . .
2',5-Cl₂	. . .	1−	. . .	. . .
3',4'-Cl₂	. . .	0	0	0
3,5-Cl₂	. . .	0+	. . .	. . .
4',5-Cl₂	++	3+	1+	1
2',4',5-Cl₃	++	2+	. . .	2+
3,3',5-Cl₃	++	3	. . .	1−
3,4',5-Cl₃	++	3+	3	1
3',4',5-Cl₃	++	3+	3+	1
2',3,4',5-Cl₄	++	4	3+	2
3,3',4',5-Cl₄	++	4	4	4
2'-Br-5-Cl	++	0	. . .	. . .
4'-Br-5-Cl	++	3+	2	1
5-Br-2'-Cl	. . .	2−	. . .	. . .
5-Br-4'-Cl	++	3−	2	. . .
2',4'-Br₂-5-Cl	. . .	4	. . .	3−
3,4'-Br₂-5-Cl	++	4−	3	. . .
3,5-Br₂-2'-Cl	++	1+	1	. . .
3,5-Br₂-3'-Cl	++	1+	1+	. . .
3,5-Br₂-4'-Cl	++	2+	3	. . .
4',5-Br₂-2'-Cl	++	3+	. . .	. . .
4',5-Br₂-3-Cl	++	4	3	. . .
3-Br-4',5-Cl₂	. . .	3+	4−	. . .
4'-Br-3,5-Cl₂	++	3	4−	. . .
5-Br-3,4'-Cl₂	. . .	3+	4−	. . .
5-Br-3',4'-Cl₂	++	4	. . .	. . .
3,4',5-Br₃-3'-Cl	++	4	. . .	3+
3,5-Br₂-2',4'-Cl₂	++	4−	3+	1+
3,5-Br₂-2',5'-Cl₂	++	3	1	. . .
3,5-Br₂-3',4'-Cl₂	++	4	. . .	4
4'-Br-3,3',5-Cl₃	. . .	4−	. . .	4−
3,4',5-Br₃-2',5'-Cl₂	++	4	4	4
3,5-Br₂-4'-F	. . .	1−	. . .	. . .
Halogenated Sulfur Derivatives of Salicylanilide				
3,5-Br₂-2'-SO₂NH₂	−	0	. . .	. . .
3,4',5-Br₃-2'-SO₂NH₂	++	1	. . .	. . .
3,5-Br₂-4'-SO₂NH₂	−	0	. . .	. . .
3,5-Br₂-2'-SH[e]	. . .	0	. . .	. . .
Halogenated Homologs of Salicylanilide				
3,4',5-Br₃-N-CH₃	−−	0	0	
4'-Br-3-CH₃	−	0	. . .	. . .
3',5-Br₂-3-CH₃	++	1	. . .	. . .
4',5-Br₂-3-CH₃	++	1	. . .	. . .
3',4',5-Br₃-3-CH₃	+	1	. . .	. . .
5-Br-2',4'-Cl₂-3-CH₃	++	3	. . .	1
5-Br-2',5'-Cl₂-3-CH₃	++	2	. . .	1−
3,5-Br₂-3'-CH₃	++	1	. . .	. . .
3,4',5-Br₃-3'-CH₃	++	4−	4	1+
3,5-Br₂-4'-CH₃	++	0	. . .	. . .
5-Br-3'-Cl-4'-CH₃	. . .	2−	. . .	0+
3,5-Br₂-3'-Cl-4'-CH₃	. . .	3+	3+	1−
5-Br-3,4'-(CH₃)₂	−−	0	. . .	. . .

TABLE III.   (*continued*)

Substituent and Position	FPD Test[a] in Presence of Bar Stock Type A[b]	SDS Test[a] in Presence of Bar Stock Type A[b]	Type B[c]	Type C[d]
Other Halogenated Salicylanilides				
5,5'-Cl$_2$-2'-OCH$_3$	++	...	0	...
4'-Br-4-NHCOCH$_3$	—	0	0	...
5-Br-3',4'-Cl$_2$-3-C$_6$H$_5$	...	2+	...	0
4',5-Br$_2$-O- Acetyl	—	...	...	...
3',4'-Cl$_2$-3-NO$_2$	++	1—	...	...
3',4'-Cl$_2$-5-NO$_2$	+	1+	...	1—
5-Br-3',4'-Cl$_2$-3-NO$_2$	++	3+	...	...
4-Hydroxybenzanilides				
4'-Br	—	...	...	...
3,4'-Br$_2$	—	0	...	...
3,5-Br$_2$	—	...	...	...
3,4',5-Br$_3$	—	0	...	...
Resorcylanilides				
4'-Br	—	0	...	...
4',5-Br$_2$	—	0	...	...
3,4',5-Br$_3$	—	0	...	...
N-Benzylsalicylamides				
3,5-Br$_2$-4'-Cl	++	1	...	...
3,5-Br$_2$-3',4'-Cl$_2$	++ (1%)	1 (1%)	...	...
Schiff's Bases				
Amine Aldehyde				
4-Amino-salicylic acid 5-Bromosalicylaldehyde	—	0	...	...
4-Bromo-aniline Salicylaldehyde	—	0	...	...
4-Bromo-aniline 5-Bromosalicylaldehyde	—	0	...	...
2-Hydroxy-aniline 5-Bromosalicylaldehyde	—	0	...	...
Other Compounds				
2-Amino-3,4',5-tri-bromobenzanilide	—	...	...	...
4,4'-Dibromo-3-hydroxy-2-naphthanilide	—	1+	...	...
4'-Bromo-2-mercapto-benzanilide[e]	—	0 (1%)	...	...
3,5-Dibromosalicyl-amide	—	...	...	...
N,N-Hexamethylenebis-(3,5-dibromosalicyl-amide)	++	0	. .	...

a See Ref. 20.   *b* Soap bar stock, pH 10.2.   *c* A soap/detergent bar stock, pH 9.2.   *d* A detergent bar stock, pH 7.0.   *e* As the disulfide.

Thus, none of the various thiol or sulfonamide derivatives showed more than moderate activity. The presence of a hydroxyl group in the 4-position destroyed activity whether or not the hydroxyl in the 2-position was retained. Esterification of the phenolic hydroxyl destroyed activity altogether as did the methylation of the nitrogen.

Simple alkylation of one of the benzene rings did not depress the test scores. Thus, 3'-methyl-3,4',5-tribromosalicylanilide was as active as 3,4',5-tribromosalicylanilide.

Of the substituents investigated only the nitro group appeared to approach the halogens in the ability to confer germicidal activity upon the salicylanilide structure. Thus, 3',4' - dichloro - 5-nitrosalicylanilide is marginally more active than the corresponding 5-chloro or 5-bromo compounds.

Structures analogous to salicylanilide such as Schiff's bases derived from salicylaldehyde, resorcylanilides, 4-hydroxybenzanilides and N-benzylsalicylamides showed little or no germicidal activity.

Table III summarizes the biological activity of the test compounds.

CONCLUSIONS

Germicidal activity and skin substantivity of halogenated salicylanilides are influenced by the test medium. Of the ninety-two compounds here

described, fewer were found to be active in the presence of a neutral detergent than in the presence of an alkaline soap.

In a soap base, high germicidal activity and skin substantivity of salicylanilides is associated with halogenation in the following positions: 4′,5; 2′,4′,5; 3,4′,5; 3′,4′,5; 4,4′,5; 2′,3,4′,5; 3,3′,4′,5; 2′,3,4′,5,5′.

In soap/detergent bar stock of intermediate pH several of the products are less active. In particular, all of the 4′,5-dihalosalicylanilides have SDS test scores of less than 3 in this medium. In neutral detergent bar stock only the 3,3′,4′,5 - tetrahalosalicylanilides and 2′,5′-dichloro - 3,4′,5 - tribromosalicylanilide exhibit appreciable activity.

Substitution of salicylanilide with groups other than halogens or modification of the salicylanilide structure usually diminishes the activity of these germicides. Only certain homologs and nitro-derivatives of the halosalicylanilides show high activity. Ring substitution with hydroxyl, methoxyl, thiol, or sulfonamide groups affects the activity adversely. Germicidal activity is destroyed completely by methylation of the amide nitrogen or by acetylation of the phenolic hydroxyl. Halogenated salicylanilides possess higher germicidal activity and skin substantivity than analogous derivatives of N-benzylsalicylamide, salicylnaphthanilides, or Schiff's bases of salicylaldehyde.

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Spectrophotometric Assay of Opium and Paregoric

By CHARLES MILOS

A method is proposed for the determination of the morphine content of opium and paregoric. The method combines extractive and spectrophotometric procedures and results in a substantial saving of time when compared to most existing methods. Results thus far obtained indicate that the method is sufficiently accurate for routine analyses.

THE DETERMINATION of morphine in opium by the U. S. P. method requires a preliminary extraction with water, evaporation of the filtrate, addition of calcium hydroxide and filtration, precipitation, and titration of the morphine (1). Most other common methods require an initial extraction of the opium with lime water and subsequent precipitation or extraction of the morphine from an aliquot of the lime water filtrate. All of these methods are long, involved, and require considerable experience to secure consistent results. There is no general agreement as to what method gives the most accurate value for the morphine content of opium. As a matter of fact, all of the methods probably have sources of error; and determinations made by different methods usually show considerable variation. Buchi, Huber, and Schumacher have